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Processing and characterization of diatom nanoparticles and microparticles as potential source of silicon for bone tissue engineering

Thi Duy Hanh Le [a,b], Walter Bonani [a,b,c], Giorgio Speranza [d], Vincenzo Sglavo [a], Riccardo Ceccato [a], Devid Maniglio [a,b,c], Antonella Motta [a,b,c], Claudio Migliaresi [a,b,c,*]

[a] Department of Industrial Engineering, University of Trento, Trento, Italy
[b] BIOtech Research Center and European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Trento, Italy
[c] Interuniversity Consortium for Science and Technology of Materials, Trento Research Unit, Trento, Italy
[d] Center for Materials and Microsystems, PAM-SE, Fondazione Bruno Kessler, Trento, Italy

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ABSTRACT

Silicon plays an important role in bone formation and maintenance, improving osteoblast cell function and inducing mineralization. Often, bone deformation and long bone abnormalities have been associated with silica/silicon deficiency. Diatomite, a natural deposit of diatom skeleton, is a cheap and abundant source of biogenic silica. The aim of the present study is to validate the potential of diatom particles derived from diatom skeletons as silicon-donor materials for bone tissue engineering applications. Raw diatomite (RD) and calcined diatomite (CD) powders were purified by acid treatments, and diatom microparticles (MPs) and nanoparticles (NPs) were produced by fragmentation of purified diatoms under alkaline conditions. The influence of processing on the surface chemical composition of purified diatomites was evaluated by X-ray photoelectron spectroscopy (XPS). Diatoms NPs were also characterized in terms of morphology and size distribution by transmission electron microscopy (TEM) and Dynamic light scattering (DLS), while diatom MPs morphology was analyzed by scanning electron microscopy (SEM). Surface area and microporosity of the diatom particles were evaluated by nitrogen physisorption methods. Release of silicon ions from diatom-derived particles was demonstrated using inductively coupled plasma optical emission spectrometry (ICP/OES); furthermore, silicon release kinetic was found to be influenced by diatomite purification method and particle size. Diatom-derived microparticles (MPs) and nanoparticles (NPs) showed limited or no cytotoxic effect in vitro depending on the administration conditions.

1. Introduction

Silicon is the main component of silica formed exo- and endo-skeletons in some marine organisms [1]. The skeleton of unicellular marine organisms such as sea sponges and diatoms consists of hydrated amorphous silica which is gradually formed by immobilization and internalization of monosilicic acid in a process addressed as biosilicification [2–4].

Nevertheless, silicon is also involved in the biomineralization processes in mammals. Calcification involves many stages including formation of calcium phosphate under the direct regulatory control of several biological systems and in the presence of elemental traces such as silicon, zinc and magnesium [5–7]. Silicon is believed to be an essential element for bone development, although its role is not completely understood [8,9]. For instance, silicon has been associated with the precipitation of calcium phosphate in the early stage of bone mineralization [7,10]. In addition, the presence of silicon at the inorganic/organic interface regulates the interaction between collagen and proteoglycans improving the quality of the extracellular matrix (ECM) [11]. Silicon can induce stem cell differentiation in osteoblasts and osteocytes [12–14]; furthermore, silicon directly inhibits osteoclast formation and bone resorption [15].

The use of degradable amorphous silica particles has been proposed to improve mineralization in bone regeneration applications besides other inorganic materials such as hydroxylapatite, tri-calcium phosphate, glass ceramic or zirconia [16,17]. However, bioactivity of particles significantly depends on size, shape and surface properties [18–21]. Recent studies have been focused on possible applications of amorphous silica nanoparticles as dietary supplement for bone regeneration [22,23]. Additionally, silica has been successfully incorporated with hydroxylapatite to enhance osteoconductivity of scaffolds for bone

Abbreviations: RD, raw diatomite powder; CD, calcined diatomite powder; AD, Acid-purified raw diatomite powder; CAD, Acid-purified calcined diatomite powder; D-MPs, Diatom microparticles; D-NPs, diatom nanoparticles; XPS, X-ray photoelectron spectroscopy; TEM, transmission electron microscopy; SAED, Selected area (electron) diffraction; SEM, scanning electron microscopy; ICP, inductively coupled plasma; EDAX, Energy dispersive X-ray analysis; DLS, Dynamic light scattering.

* Corresponding author at: Department of Industrial Engineering, University of Trento, Via Sommarive 9, 38123 Trento, Italy.
E-mail address: claudio.migliaresi@unitn.it (C. Migliaresi).

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tissue regeneration [24,25]. Silk or collagen constructs loaded with amorphous silica particles have been successfully proposed to improve osteoinductivity [26–28]. So far a variety of amorphous silica sources have been considered. Often silica particles are of synthetic origin and are produced using chemicals and surfactants whose residues might have toxic effects [18,29]. So, there is a quest for abundant and reliable alternative sources of amorphous silica.

Diatomite, also known as diatomaceous earth, is the marine sediment of silica diatom skeleton remains. Diatomite is an inexpensive and unlimited source of biogenic silica. Thanks to their peculiar morphology and porosity, diatom skeletons derived from diatomite have been proposed for uses in photonics, drug delivery and molecular catalysis applications [30–33]. We think that diatomite could be a promising natural source of amorphous silica and biogenic silica also for bone tissue engineering applications. Biomedical uses of biogenic silica have been preconized by Wang et al. [2], but to date diatomite-derived silica particles have never been used as a part of tissue engineering scaffolds. We believe that diatom microparticles and nanoparticles could be useful as bioactive silicon-donor additives for degradable engineered scaffolds and bone defect fillers following the silicon role on bone mineralization.

However, raw diatomite contains some local contaminations such as clays and other inorganic and organic compounds that require purification before any medical use and the yield of diatomite purification processes depends on diatom type and source [34].

Here, raw diatomite (RD) and calcined diatomite (CD) powders were purified in strong acid conditions, and diatom nanoparticles (NPs) and microparticles (MPs) were subsequently produced by treating the skeletons in alkaline solution. NPs and MPs morphology, elemental composition and specific surface area were determined. Silicon ion released by diatom particles dissolution has been evaluated with dissolution experiments and cytotoxicity tests of diatom particles have been performed.

2. Materials and methods

2.1. Materials

Powder form of raw diatomite materials (RD) used in this study was provided by Phu Yen mineral joint stock company (Phu Yen province, Viet Nam). RD powder was passed through a metallic sieve (mesh size 250 μm) to remove aggregates and macroscopic contaminations.

Phosphate buffer solution (PBS), sodium hydroxide (NaOH), hydrochloric acid (HCl) and Triton X-100 were purchase from Sigma-Aldrich (St. Louis, MO, USA). All reagents and solvents were used as received without further purification.

2.2. Raw diatomite purifications

2.2.1. Acid-purified raw diatomite powder

RD powder underwent acid treatment to remove inorganic contaminations; purification protocol modified from [35]. Briefly, RD powder was dried overnight in oven at 102 °C, passed through a metallic sieve (mesh size 125 μm) to remove larger aggregates, and then acid-treated with 1 M HCl solution at 55 °C (in the proportion of 100 mg of powder per ml of HCl solution) for 24 h under continuous stirring to remove the inorganic contamination. Afterwards, the obtained slurry was concentrated with a paper filter; the remaining solid part was washed and allowed to sediment in deionized water (DI water) for at least 10 times. Finally, the sediment was dried in oven at 102 °C and sieved through a 63 μm pore size sieve to obtain acid-purified RD (hereinafter AD) consisting of single diatoms.

2.2.2. Acid-purified calcined diatomite powder

In this case, raw diatomite powder (RD) was heated at 650 °C in air for 3 h to reduce organic contaminations [36]. Calcined diatomite powder (CD) was then passed through a metallic sieve (mesh size 125 μm), then treated with acid as explained before to obtain acid-purified CD (hereinafter named CAD).

2.3. Diatom microparticles and nanoparticles from purified diatoms

Diatom microparticles and nanoparticles were produced from purified diatom powders (both AD and CAD) by mechanical fragmentation in alkaline conditions [37,38]. Briefly, AD and CAD powders were suspended in 0.1 M NaOH solution (typically, 10 mg of diatomite powder per ml of alkaline solution was used), and suspension was vigorously stirred for 2 weeks at room temperature (RT) to break diatoms. Afterward, the alkaline suspension was kept at RT for one week to allow for sedimentation.

The unsettled colloidal suspension was collected separately and centrifuged at 15,000 rpm for 30 min to retrieve diatom nanoparticles (here named AD-NPs and CAD-NPs). The obtained NPs were subsequently washed in DI water and centrifuge (15,000 rpm for 30 min) for 3 times to remove any NaOH traces. The settled solid fraction was also collected, re-suspended in DI water and centrifuge as above to recover trapped NPs.

Finally, the remaining settled fraction was collected and washed with DI water to obtain diatom microparticles (named AD-MPs and CAD-MPs, depending on the source of purified diatomite).

2.4. Diatomite, purified diatomite and diatom particles characterization

Composition and mineral contamination of the RD powder, AD and CAD purified powders were characterized by X-ray diffraction (XRD) with a high resolution powder diffractometer (Rigaku PMG/VH, Tokyo, Japan), with Bragg–Brentano geometry in the range 2θ from 5.0–60.0° using CuKα radiation ($\lambda = 1.5405981$ Å). Surface atomic composition of diatomite powders before and after purification was analyzed by X-ray photoelectron spectroscopy (XPS) with a Scienta Gammadata ESCA 200 (Uppsala, Sweden), equipped with monochromatic Al-Kα radiation source ($h\nu = 1486$ eV).

A Field-Emission scanning electron microscope (FESEM-Supra 40, Zeiss, Germany) was used for the observation of diatomite powders, diatoms morphology and microparticles size distribution using type II secondary electrons (SE2).

Back scattered electrons (BSE) combined with Energy-Dispersive X-ray analysis (EDAX) were used to detect elemental composition of diatom and contaminations using a FEI/Philips XL30 Environmental Scanning Electron Microscope (ESEM, FEI, Hillsboro, Oregon, USA) equipped with Falcon X-Ray Microanalysis System.

The hydrodynamic radius of diatom NPs in both DI water and PBS was determined by Dynamic Light Scattering (DLS) using a Malvern 110 Zetasizer Nano ZS instrument (Malvern, United Kingdom), equipped with a He–Ne (a 5 mW laser at 633 nm). Morphology and chemical analysis of the NPs were also confirmed by transmission electron microscopy with a CM12 TEM, (Philips, Eindhoven, Netherlands) – accelerating voltage 120 keV – combined with Energy Dispersive X-Ray spectrometer (EXDS).

Surface area and pore size distribution microparticles and nanoparticles were evaluated by physisorption measurements. Nitrogen physisorption experiments were performed at the liquid nitrogen temperature using a Micromeritics ASAP 2010 system (Norcross, GA, USA). All the samples were degassed below 1.3 Pa at 25 °C prior to the measurement. The Specific Surface Area (SSA) values were calculated by the BET equation in the interval $0.05 \le (p/p_0) \le 0.33$ [39]. Pore size distribution was calculated using the BJH method applied on both branches of the physisorption isotherms [40].